ABSTRACT

Agents and methods for the diagnosis and therapy of Alzheimer's disease are disclosed. Such agents include three genes located within the region of human chromosome 21 occupied by the APP gene, which are exclusively expressed in Alzheimer's disease, respectively, the proteins encoded and expressed by these genes, the nucleic acid molecules influencing their expression, and endogenous antibodies produced in humans with Alzheimer's disease against the above proteins. Also disclosed are antibodies and nucleotides to detect the presence of the proteins and nucleic acids in humans.

Figure legends:

Figure# 1

ATG = open reading frame
* = stop translation signal
P = poly A addition signal
pck = protein kinase phosphorylation site
ck = casein kinase phosphorylation site
downwards pointing arrow = secretory/cleavage site
single overline = cap site
double overline = GC promoter element
double overline and underline = CCAAT promoter element
single underline = TATA promoter element
LxxxxxxL = leucine zipper
ooooooo = heat shock element (HSE)
[ ] = transmembrane signal
xxxxx = iron binding site
[C/H--C/H].....[C/H--C/H] = potential zinc finger
= estrogen responsive elemeent (ERE)

Figure # 2

= promoters

atg = orf
* = stop translation signal
p = poly A addition signal
—— + —— = size of fragments expected if potential cleavage site is used
= sequence in alzas identical to sequence in ALZASp.
i = intron
e = exon

Figure 3

PCR amplification:

A. mRNA isolated from postmortem AD and non AD tissues using alz 287 primer pair lane 1 = DS brain, lanes 2-3 = normal brain; lane 4 = AD cortex; lane 5= AD cerebellum; lane 6 = DNA markers; 7= normal lymphocyte; 9 = AD lymphocyte; 10 = DNA markers
B. using primer pair alz 148, lane 10&13 = DNA markers; lane 11 = AD cortex; lane 12 = normal brain

Figure 4

A = description of antigen trap ELISA test
B = description of antibody trap ELISA test
C = Dot plots of ELISA values for endogenous anti-ALSAS IgG measured in serum from 34 autopsy confirmed AD victims and 15 normal subjects. The values were not corrected for the normal background of the test.
(D) Box plots of ELISA values for endogenous anti-ALZAS IgG measured in serum from patients diagnosed with depression, patients diagnosed with clinical AD, and normal controls (the normal serum were obtained from subjects involved in a pre symptomatic breast cancer detection study).
(E)Dot plots of ELISA values for anti.ALZAS endogenous antibody measured in serum obtained from normal control subjects, clinically diagnosed and autopsy confirmed AD positive controls (mixed) and and from subjects over 65 years old who have no clinical symptoms of AD, selected at random from available sera (screening).

Figure 5A-D

(A). Isolation of ALZAS from cortex obtained from a single autopsy confirmed AD patient. The protein was affinity purified on anti ALZab2 antibody linked to CNBR-sepharose. The gel was blotted onto nylon a nylon membrane and the protein detected with a chemiluminescent detection system.
(B) Silver stain of SDS gel: proteins obtained following affinity purification of ALZAS from serum from a late stage AD patient. In addition to ALZAS, ALZAS bound to various immunoglobulins and to APOE was detected on the silver stained gel and confirmed by specific staining duplicate gels
(C) Detection of ALZAS-IgG complexes present on gel of 5B with anti-human IgG (Fc fragment).
(D) Western blot of cationic non SDS polyacrylamide gel following electrophoresis of serum from (a) a patient with sporadic AD, and (b) a patient with swedish mutation AD. The gels were reacted first with anti ALZASab1 and then with the chemiluminescence system. ALZAS was detected in a and ALZAS2 in b. The upper band in a is ALZAS and in b is ALZAS2 both bound to human IgG. ALZAS and ALZAS2 have slightly different isoelectric points which are consistent with how they appear on the gels.

Table 1A

PCR primers used to detect mRNA in lymphocytes and brain

alzas 287	product size expected =	287 bp
Forward	GTGGACAAATATCAACACCGAGGAC	
Reverse	ACATAGTCTTAATTCCCACTTGG	
alzas 393	product size expected =	393 bp
Forward	GTCCTGCATACTTTAATTATGATG	
Reverse	AGCCATCATGGAAGCACACTGATTCG	
alzas 188	product size expected =	188 bp
Forward	GTGGACAAATATCAAGACGGAGGAG	
Reverse	TCCTTAATTTGATTTCTAGCACAGG	
alzas 267	product size expected =	267 bp
Forward	TCCTGCATACCTTTAATTATGATG	
Reverse	TTCATGGTAATCCTATAGGCAAC	
alzas 148	product size expected =	148 bp
Forward	GTGTTCTTTGCAGAAGATGTGGG	
Reverse	ACATAGTCTTAATTCCCACTTGG	